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TOXICITY OF 19 ADJUVANTS TO JUVENILE *LEPOMIS MACROCHIRUS* (BLUEGILL SUNFISH)

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Abstract—Nineteen adjuvants, many used as surfactants for aquatic herbicide applications, were applied in static bioassay to bluegill sunfish (*Lepomis macrochirus*) for 96 h to determine median lethal concentrations (LC50). Surfactants are added to the tank mix as a percentage (v/v) of the total volume, in contrast to herbicide application rates, which are usually expressed in kilograms per hectare. Two ethoxylated tallow amine products were the most toxic, having LC50 values of 1.6 and 2.9 ppm (all values v/v). Seven alcohol/glycol-based surfactants had 96-h LC50 values of 4.0 to 11.6 ppm (mean = 7.9 ppm). The polysiloxane- or silicone-based surfactants had toxicities of 18.1 to 29.7 ppm (mean = 24.7). Two limonene-based products had LC50 values of 10.2 and 30.2 ppm. A methylated seed oil with emulsifier had a LC50 of 53.1 ppm. Two acid/buffer utility adjuvants had LC50 values of 60.8 and 221 ppm. To compare the relative safety of the tested surfactants, we assumed maximum label rate applications to 1 m deep water with uniform mixing. This comparison of relative safety is based on mortality to 50% of the test organisms and does not imply application rates that would not result in any mortality. The two ethoxylated tallow amines, neither used or recommended for aquatic applications, had a relative safety factor of 12.6 or less. Relative safety factor varied from 6.2 to 20.4 for the seven alcohol/glycol surfactants, 38.4 to 63.2 for silicone-based products, 5.5 to 16.1 for limonene products, 113 for methylated seed oil, and 132.2 to 315.7 for acid/buffer utility adjuvants. When used according to label recommendations under normal use conditions, these adjuvants should not be present in acutely toxic concentrations; however, the most toxic adjuvants in very shallow water (<10 cm) would be toxic to bluegill sunfish that did not move to deeper water to avoid lethal concentrations.

Keywords—Surfactants   Ethoxylated tallow amine   Alcohol/glycol   Polysiloxane/silicone   Herbicides

INTRODUCTION

Adjuvants are chemicals or, more commonly, a combination of chemicals added to facilitate or modify a pesticide spray mixture to improve ease of application or increase pesticide effectiveness. Surface active agents (surfactants) are widely used in soaps, cosmetics, pharmaceuticals, and pesticide application. Older surfactants (e.g., soap, whale oil) were used in the early 1900s to reduce surface tension of water and increase wettability and thus penetration of foliar-applied herbicides. Surfactant use in agriculture became widespread in the early 1960s because of improved efficacy when added to triazine and urea herbicides [1].

The stimulus to increase awareness and use of surfactants in aquatic weed control occurred in the early 1980s when glyphosate (Rodeo, all manufacturers and locations for commercial products are given in Table 1) was registered for aquatic use by Monsanto (St. Louis, MO, USA). The commercial Rodeo formulation did not contain sufficient surfactant for optimum efficacy, and the label specifically instructed aquatic applicators to add nonionic surfactants of the applicator's choice. Aquatic applicator interest in adjuvants for drift control, sinking agents, rain fastness, inverting oils, foam/dye markers, and improved herbicide efficacy led to testing and evaluation of many new adjuvants at that time, paralleling increased surfactant use in agriculture [2].

In 1985, the acute toxicities of adjuvants commonly used in aquatic sites were evaluated [3]. Surfactant use in aquatic plant management has since increased, new surfactants have been formulated (silicone and methylated seed oils), and new uses have been developed (e.g., addition of surfactants to contact herbicides for improved submersed weed control).

Pesticide adjuvants are formulated with chemicals and products listed by the U.S. Environmental Protection Agency or Food and Drug Administration (http://www.epa.gov/opprd001/inerts). Although extensive testing is not required for these exempt compounds, manufacturers of adjuvants typically have proprietary data that are not widely available or standardized. Fish and aquatic invertebrate toxicities for several adjuvants, and particularly for adjuvant components, have been published.

Dorn et al. [4] evaluated the effects of a $C_{12–13}$ linear alcohol ethoxylate surfactant on several aquatic organisms in mesocosm tests. The rates of linear alcohol ethoxylate surfactant evaluated over a 30-d period were 0.32, 0.88, 1.99, and 5.15 ppm. Periphyton and aquatic vascular plants were not affected at any rates tested, whereas insects (Simuliidae) and zooplankton were significantly reduced at the lowest linear alcohol ethoxylate surfactant rates evaluated (0.32 ppm). The 30-d LC50s (concentration at which 50% of test organisms died) for *Pimephales promelas* (fathead minnow) and *Lepomis macrochirus* (bluegill sunfish) were 1.27 and 1.30 ppm, respectively.

Swedmark et al. [5] evaluated the effects of five surfactant components on selected marine animals. Fish were most susceptible, having 96-h LC50 values of 0.8 to 6.5 ppm, followed by bivalves at 5 to >100 ppm, while crustacean toxicities varied from 25 to >100 ppm. This general relationship is supported by Abdelghani et al. [6], who found that Syndets surfactant (80% polyethylene glycol alkyl ether and 20% acids)

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Table 1. Adjuvants, producers, ingredients, and use rates for products used to determine the median lethal concentration (LC50) to juvenile *Lepomis macrochirus* (bluegill sunfish)

Product name	Trademark holder	Class	Ingredients as listed on product label[a]	Aquatic use directions on label	Maximum use rate[b] (L/ha)
MON 0818	Monsanto Co. (St. Louis, MO, USA)	Tallow amine	Ethoxylated tallow amine 68–73% per material safety data sheet	No	Experimental use
Entry II	Monsanto Co.	Tallow amine	35% ethoxylated tallow amine	No	2.3
SA-50	Southern Agricultural Insecticides (Hendersonville, NC, USA)	Alcohol/glycol	80% alkyl aryl polyoxyethylene glycol and other ethoxylated derivations	No	4.7
X-77 Spreader	Loveland Industries (Greeley, CO, USA)	Alcohol/glycol/silicone	90% alkylarglypolyoxyethylene, alkylpolyoxyethylene, fatty acids, glycols, and dimethylpolysiloxane	Yes	4.7
Aqua-King	Estes Incorporated (Wichita Falls, TX, USA)	Alcohol/glycol	90% alkylphenol-hydroxypolyoxyethylene, glycols, and isopropanol	Yes	4.7
Optima	Helena Chemical Co. (Memphis, TN, USA)	Alcohol/glycol	90% polyethoxylated alkylamines (C_8–C_{18}), alkylpolyoxyethylene glycols, and organic acids	No	9.3
Induce	SETRE Chemical Co. (Memphis, TN, USA)	Alcohol/glycol	90% alkylarylpolyoxylkane ether and free fatty acids	Yes	4.7
Timberland 90	Timberland Enterprises (Monticello, AR, USA)	Alcohol/glycol	90% alkyl aryl polyoxyethylene ether, isopropanol, and free fatty acids	No	4.7
Cide-Kick	Brewer International (Vero Beach, FL, USA)	*d′*-limonene	100% *d′*-limonene and related isomers plus selected emulsifiers	Yes	18.7
Big Sur 90	Brewer International	Alcohol/glycol	90% alkylaryl polyoxyethylene glycols, free fatty acids, and isopropanol	Yes	18.7
Sil Energy	Brewer International	Silicone	99% polyalkyleneoxide-modified polydimethylsiloxane and nonionic surfactants	Yes	4.7
Kinetic	SETRE Chemical Co.	Silicone	99% polyalkyleneoxide-modified polydimethylsiloxane and polyoxypropylene-polyoxyethylene block copolymers	Yes	4.7
Dyne-Amic	Helena Chemical Co.	Silicone	99% polyalkyleneoxide-modified polydimethyl siloxane, nonionic emulsifiers, and vegetable oils	Yes	7.0
Thoroughbred	Estes Incorporated	Silicone	99% polyalkyleneoxide-modified polydimethylsiloxane and nonionic surfactants	Yes	4.7
Freeway	Loveland Industries	Silicone	100% silicone-polyether copolymer and alcohol ethoxylates	No	4.7
Cygnet Plus	Cygnet Enterprises (Flint, MI, USA)	*d′l′*-limonene	100% *d′l′*-limonene and related isomers plus selected emulsifiers	Yes	18.7
Sun Wet	Brewer International	Methylated seed oil	100% blend of methylated seed oil and emulsifiers	No	4.7
LI 700	Loveland Industries	Acid/buffers	80% phosphatidylcholine, methylacetic acid, and alkyl polyoxyethylene ether	No	4.7
Quest	SETRE Chemical	Acid/buffers	50% ammonium salts of polyacrylic and hydroxy carboxylic and phosphoric acids	Yes	7.0

[a] Percentages are of the commercial formulation as sold as indicated on the product label.
[b] Directions for applying surfactants in aquatic habitats are given as liters per hectare (pints/acre), or as a percentage (0.25–0.50%) of the total volume applied per hectare (acre); maximum rates are shown as liter per hectare assuming an application volume of 935 L/ha (100 gal/acre).

was toxic (LC50) to bluegill sunfish at 1.9 ppm, catfish at 2.3 ppm, and crawfish at 15.2 ppm.

Parr [7] and Lewis [8] have attempted to summarize surfactant toxicities of the literally thousands of surfactants, detergents, emulsifiers, and other adjuvants available commercially. Cationic surfactants are, in general, more toxic to plants and animals than anionic or nonionic surfactants.

Rapid changes have occurred in agricultural adjuvant use over the past decade [9]. The compendium of herbicide adjuvants first published by Kapusta in 1992 [10] listed 76 adjuvants manufactured by 22 companies, whereas the fifth edition of the compendium published by Young in 2000 [11] lists 391 entries from 36 companies. Young postulated that increased adjuvant use in agriculture was due to increased use of foliar-applied herbicides, more reliable research showing benefits of adjuvant use, and development of mixes containing multiple components to address requirements of specific herbicides.

This increased use of adjuvants prompted a study of 19 adjuvants, primarily nonionic surfactants used in aquatic weed

Table 2. Adjuvants and their median lethal concentration (LC50) to juvenile *Lepomis macrochirus* (bluegill sunfish) in 96-h static bioassays and relative safety factor calculated from maximum label rat and LC50 values (assumes application to 1-m–deep water with uniform mixing; since LC50 values represent the concentration at which 50% of test organisms died, the relative safety factor does not imply that concentrations cannot be damaging to organisms)[a]

		Toxicity		Maximum rate application effects	
Product name	Class	LC50 (ppm)	95% confidence interval (ppm)	Expected concn. (ppm)	Calculated relative safety factor
MON 0818	Tallow amine	1.6	1.5–1.7	NA[b]	NA
Entry II	Tallow amine	2.9	2.7–3.0	0.23	12.6
SA-50	Alcohol/glycol	4.0	3.5–4.4	0.47	8.5
X-77 Spreader	Alcohol/glycol/silicone	5.0	4.7–5.3	0.47	10.6
Aqua-King	Alcohol/glycol	7.4	6.9–7.9	0.47	15.7
Optima	Alcohol/glycol	8.6	7.9–9.2	0.93	9.2
Induce	Alcohol/glycol	9.0	8.2–9.8	0.47	19.1
Timberland 90	Alcohol/glycol	9.6	8.8–10.3	0.47	20.4
Cide-Kick	*d′*-limonene	10.2	9.1–11.3	1.87	5.5
Big Sur 90	Alcohol/glycol	11.6	11.0–12.1	1.87	6.2
Sil Energy	Silicone	18.1	16.6–19.5	0.47	38.5
Kinetic	Silicone	19.8	18.7–21.0	0.47	42.1
Dyne-Amic	Silicone	26.9	25.5–28.3	0.70	38.4
Thoroughbred	Silicone	28.9	26.5–31.3	0.47	61.5
Freeway	Silicone	29.7	28.7–30.7	0.47	63.2
Cygnet Plus	*d′l′*-limonene	30.2	29.0–31.5	1.87	16.1
Sun Wet	Methylated seed oil	53.1	51.1–55.1	0.47	113.0
LI 700	Acid/buffers	60.8	57.9–63.8	0.47	132.2
Quest	Acid/buffers	221	203–239	0.70	315.7

[a] See Table 1 for name and location of manufacturers.
[b] NA = not applicable, experimental product.

management, to determine acute toxicities to juvenile bluegill sunfish.

METHODOLOGY

A static 96-h bioassay for acute toxicity was conducted in a temperature-controlled room (20 ± 2°C) for 19 adjuvants (Table 1) using juvenile bluegill sunfish following the procedures outlined by standard methods [12]. The fish were purchased locally and had been raised in earthen ponds. On delivery, fish were held for 2 to 4 d in aerated 1,000-L tanks at the Department of Fisheries and Aquatic Sciences Aquaculture Facility (University of Florida, Gainesville, FL, USA). Subsamples of the fish used in the trials were collected during the trials (fall 1999–winter 2000). Fifty-six randomly selected fish varied from 36 to 51 mm (mean = 42.0, standard error of the mean [SE] = 0.4) total length and weighed from 0.4 to 1.5 g (mean = 0.8, SE = 0.03).

Bioassays were conducted in 15-L plastic aquaria containing 7 L of aerated well water that had been held in the facility for at least 24 h to equilibrate and aerate. Aquaria were aerated during tests. A continuously flowing (100-m deep) well supplies water to the aquaculture facility at rates exceeding 50 L/min. Appropriate amounts of test adjuvants were added to the aquarium water via pipette and thoroughly mixed prior to the introduction of fish. No further additions of adjuvant were made, nor were concentrations of adjuvant in the aquaria measured during the toxicity testing. Dissolved oxygen, temperature, and pH were monitored in the test aquaria using a YSI Scientific Model 54 oxygen meter (Yellow Springs, OH, USA) (oxygen and temperature) and a Fisher Scientific accumet portable AP10 pH meter (Fair Lawn, NJ, USA).

The static bioassays consisted of 10 fish/aquarium (1.1 g/L loading rate), with three replications per adjuvant concentration or control. Dead fish were removed daily. If mortality in the control aquaria exceeded 10% (3) of the total fish (30) in the replicate controls, the study was terminated and reestablished. Fish were used in only one test. Fish were fed commercial fish chow in the holding tanks until 24 h before tests but were not fed afterward.

Data were subjected to probit analysis to calculate LC50 values using Toxstat software (Western Ecosystems Technology, Cheyenne, WY, USA). The data are reported as LC50 (ppm on a v/v basis; e.g., 0.7 ml of commercial product in 1 m^3 of water = 0.7 ppm) and based on the formulation of the material as sold, not corrected for different concentrations of the active ingredients. Calculations were based on the formulation as sold because most are mixtures with unknown emulsifying agents and other components, making it impossible to partition toxicity to an individual active ingredient. In addition, these are applied on a liter-per-hectare basis, and the weights of the active ingredients are not provided on the labels.

RESULTS AND DISCUSSION

Oxygen concentrations were between 85 and 100% saturation in all test solutions. The well water used in these tests had a pH of 8.6, a hardness of 176 mg/L, and a total alkalinity of 162 mg/L.

Monsanto's MON 0818 and Entry II are 68 to 73% and 35% ethoxylated tallow amine surfactants, respectively, that have been used in glyphosate formulations. The material safety data sheet for MON 0818 lists 96-h toxicity to bluegill sunfish at 1.3 ppm, similar to the 1.6-ppm LC50 obtained in this study (Table 2). Entry II, with about half the active ingredient as in MON 0818, had a fish toxicity of about half that of MON 0818 (2.9 and 1.6 ppm, respectively).

The 96-h LC50 to bluegill sunfish was determined for seven products we classified as alcohol/glycol surfactants: SA-50, X-77, Aqua-King, Induce, Timberland-90, and Big Sur 90, plus Optima, classified by its manufacturer as a translocating agent consisting of surfactants and buffering agents. These surfactants contain 80 to 90% active ingredients but differing specific alcohols/glycols in differing ratios. Nevertheless, the LC50 values were fairly similar, varying from 4.0 ppm for SA-50 to 11.6 ppm for Big Sur 90. The mean for all seven products was 7.9 ppm.

The next set of surfactants that seemed to form a group among the 19 products studied were the silicone-based products. Sil Energy, Kinetic, Dyne-Amic, Thoroughbred, and Freeway had 96-h LC50 values between 18.1 (Sil Energy) and 29.7 ppm (Freeway), with a mean of 24.7 ppm. These products listed active ingredients of 99 to 100% but contained a variety of components, including vegetable oils, alcohol ethoxylates, and copolymers.

The two limonene-based products, Cide-Kick and Cygnet Plus, had quite different LC50 values of 10.2 and 30.2 ppm, respectively. The manufacturer indicated (S. Brewer, Brewer International, Vero Beach, FL, USA, personal communication) that this difference was due to the different emulsifiers in the two products. The LC50 for Cide-Kick determined in this study was 10.2 ppm, whereas data provided by Brewer International for an environmental impact assessment [13] showed a LC50 to bluegill sunfish of 18 ppm (range 2.1–58.5 ppm).

The remaining three products tested were 100% methylated seed oil with emulsifiers (Sunwet), which had a LC50 of 53.1 ppm, and two acid/buffer utility adjuvants, LI 700 and Quest. In addition to sequestering agents and acids for chelation of carbonates and pH reduction, LI 700 also contains polyoxyethylene ether. The toxicity of LI 700 was 60.8 ppm, while Quest had a LC50 of 221 ppm.

Only LI 700 and Quest resulted in different pH values in the test solutions. The highest test concentration of LI 700 (80 ppm) had a pH of 8.1, whereas Quest, at 500 ppm, reduced the pH from 8.6 to 7.2. The normal use rate of these products (0.125–0.25% v/v) in a tank mix would produce adjuvant concentrations of 1,250 to 2,500 ppm.

The LC50 values found in this study are similar to proprietary data provided by the manufacturers or other reported results that were located in the literature. Discrepancies existed with previous data that we reported in 1985 [3]. In 1985, we reported a LC50 for Cide-Kick of 5.2 ppm compared to 10.2 ppm in the current study. Likewise, we reported a Big Sur LC50 of 112 ppm in 1985 compared to Big Sur 90 at 11.6 ppm in the current study. The manufacturer indicated that changes had been made in both formulations and particularly to Big Sur, which was reformulated since 1985 (S. Brewer, Brewer International, personal communication). Data collected in this study for X-77 (LC50 5.0 ppm) suggest that the composition of X-77 may not have been changed significantly since 1985 (LC50 5.5 ppm).

As was the case in 1985 [3], adjuvant toxicity ranged widely, from 1 to 2 ppm to over 200 ppm. The most toxic material, the tallow amine MON 0818, is an experimental product. Toxicity of the remaining products under field conditions must be considered in relation to the recommended application rates. Surfactants are added as a percentage (v/v) of the total volume of the tank mix or on a liter-per-hectare basis. To compare the relative safety factor of the tested surfactants, we assumed maximum label rate for aquatic use or terrestrial use if aquatic directions were not listed on the label (Table 1). Additional assumptions were application to 1-m-deep water and uniform mixing. All evaluated adjuvants have a 5× or greater relative safety factor (Table 2) for juvenile bluegill sunfish (levels of the product applied to 1-m-deep water would have to be five or more times higher than the maximum recommended use rate to kill 50% of the bluegill sunfish). Management of emergent invasive plants such as purple loosestrife (*Lythrum salicaria*) in shallow wetlands is becoming more common as these species spread. Applications in shallow water, particularly water only a few centimeters in depth, could pose risks. However, some portion of the herbicide/adjuvant mixture would be retained on target plants, increasing the relative safety factor for applications to floating and emergent plants. Overspraying, spraying unvegetated areas, or rainfall soon after application in shallow waters (<10 cm) could result in acute toxicity to bluegill sunfish with some of these adjuvants.

Many other organisms, including some that may well be more sensitive to these products, could be used in toxicity tests such as those conducted here. It is also very possible that larval stages of bluegill sunfish would be more sensitive than the juvenile stages tested. The water used in this study, while typical of water in many Florida lakes, is higher in pH, alkalinity, and hardness than many water bodies found in other locations. It is not known how more moderate water quality parameters would affect the results. It is possible that while the absolute toxicity could change, the relative ranking among the tested adjuvants might not. Other factors that influence toxicity tests include the breakdown of surfactants during the test. Sublethal effects on behavior, reproduction, and so on were not monitored during this study and could have significant consequences to resident animal life. It is also recognized that LC50s, by their very definition, indicate mortality of 50% of a population. Any mortality rate above zero would be unacceptable in many locations, especially considering the presence of rare, threatened, or endangered organisms. Finally, longer-term (chronic) tests could show effects not found in acute toxicity testing.

While toxicity of adjuvants has not been a focus of concern for aquatic applications, the data reported here will give resource managers guidance into the acute toxicities of some of the commercially available adjuvants and assist in the development of invasive plant management programs with an acceptable margin of safety.

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